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THE BOTANICAL REVIEW

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UNSTABLE GENES

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The rediscovery of Mendel's papers[1] marks the beginning of the modern science of genetics. Since in these papers a definite gene concept was formulated, the origin of that concept may properly be placed at the same time as that of genetics although the term gene was not introduced until several years later (45).

The gene, as the unit of heredity, has attracted the attention of investigators since the early stages of genetic research. In spite of great interest in the gene problem and the large amount of work done on it, no method for direct study of gene properties has as yet been discovered. The closest approach to this was made by the recent discovery that changes in genes may be induced experimentally by x-rays and related radiations.

All our knowledge concerning the gene, therefore, is based upon observations made on visible effects of gene activity, which we have good reason to believe are not the primary ones. Changes in genes are manifested in certain characters in the organism and these characters, therefore, make possible a study of the behavior and characteristics of the genes. Hence the study of gene changes as seen in visible effects upon the organism becomes the most important method for study of the nature of the gene. This review will be devoted to results obtained in such studies and particularly to studies on unstable genes.

Descriptions of two typical cases determined by unstable genes. As an illustration of the behavior of unstable gene characters I shall give a short description of a simple case involving the variegated-rose of *Delphinium* (24) and another involving unstable miniature-3 character of *Drosophila virilis* (17). These cases are

[1] These two papers by the Austrian monk, Gregor Mendel, were published in 1866 and 1870, respectively, but did not receive much recognition until 1901 when the first of them was translated and published in English.—Editors.

selected as examples because they are simple and will be referred to again.

Flowers of the rose-variegated race of *Delphinium* show purple spots on rose background. These spots are interpreted as due to changes in the rose gene from rose into its purple allel.[2] Each of the spots, therefore, registers one such change which occurred sometime during development of the flower. If the change occurs early in development, the cell with the changed gene will have an opportunity to divide many times and, therefore, to produce a large spot; if the change occurs late in development, a small spot will be produced. Thus, size of spots indicates the time in ontogeny when the change occurred and number of spots shows frequency of changes. From seeds of a self-pollinated variegated plant, a few purple plants are obtained in addition to variegated offspring. These purple plants are the result of a change of the gene for rose into the gene for purple affecting the germ-cells. In *Delphinium,* offspring of seeds from such branches does not differ from offspring of seeds from variegated branches since color of flowers and germ-cells are located in two different tissues which separate very early in ontogeny, even before the change in the gene affecting a whole branch occurs. Flower color of *Delphinium* is present in only one epidermal layer of cells whereas germ-cells develop from sub-epidermal tissues. In some other color variegations, however, such as in variegated pericarp of maize (30), variegated flowers of *Antirrhinum* (64), in chlorophyll variegations and unstable morphological characters the germ-cells are affected by a change which covers a large enough area to include the region in which the flowers are located. In these cases, apparently, the germ-cells and the tissues showing unstable characteristics are derived from the same layer of cells; the gametes on the reverted branches are affected by the change since these gametes originate from the same tissue as the cells which show up the change in the phenotype.[3]

In its behavior, the unstable miniature character of *Drosophila*

[2] An *allelomorph* (allel) is one of two dissimilar factors which on account of their corresponding positions in corresponding chromosomes are subject to alternative inheritance (Darlington).—Editors.

[3] *Phenotype* refers to the external appearance produced by the reaction of an organism of a given *genotype* with a given environment. *Genotype* is the kind of type or the hereditary properties of an organism. (Darlington). —Editors.

virilis is similar to the variegated-rose character of *Delphinium*. A portion of the offspring of the parents carrying unstable miniature-3 gene is miniature, a portion of them has mosaic wings consisting of wild-type and of miniature tissues, and a few of them have wild-type wings. In this case the miniature gene is reverting to its wild-type allel. Wild-type regions on mosaic wings, therefore, are results of reversions which occurred during development of the wing, and the wild-type flies are reversions which affect the germ-cells.

Direction of changes in unstable genes. The important characteristics of unstable genes are that the prevalent changes in these genes go in one direction only and that these changes, in a great majority of cases, occur from a recessive[4] to a dominant allel which, in all but one known case, may be considered the wild-type allel of that unstable gene. For example, the gene for unstable white pericarp of maize changes into its dominant red allel (30), the gene for rose and lavender of *Delphinium* into the purple allels (24), for dwarf of *Portulaca* into the wild-type (8), genes for contracted, cream, delicate, dotted, flecked, lobless, miniature, pineinebustant, pupy, purple, speckled, wrinkled, yellow-inconstant and yellowy of *Pharbitis* all change into their respective wild-type allels (44).

The case of the unstable willow-leaf of *Pharbitis* (43, 44) is the only one known to me wherein the gene changes into a dominant allel which is not the wild-type allel. As shown by Imai, the willow-leaf gene is unstable both in somatic and in germinal tissues and changes into the maple-leaf allel which is dominant to willow-leaf but recessive to the wild-type.

Another possible exception to what seems to be a general rule among unstable genes is the crispa case of *Antirrhinum* (7). Crispa is a dominant character, lethal when homozygous,[5] showing as dried out lesions especially on the edges of leaves. Baur found

[4] For every character of a sexually produced organism a gene or factor is ordinarily contributed by each parent. If each gene of such a pair is different from the other, as red and white rather than red and red, and if only one of them is visibly expressed, that one is said to be *dominant* over the other which is then *recessive*.—Editors.

[5] When both factors contributed by both parents affect a certain character in the progeny exactly alike, as red color from each parent, the offspring is said to be *homozygous* for that characer. If the inherited genes or factors are unlike, as red and white, the offspring is *heterozygous*.—Editors.

that a strain, which had been tested in large numbers for three generations, produced about two per cent. crispa plants. He concluded that the wild-type allel of the crispa locus of that strain frequently changes into the crispa allel, that it is in an unstable condition. In this case, however, the possibility is not excluded that the crispa character is formed by some other mechanism than a gene mutation. Only one out of several such mechanisms will be mentioned here. If the strain throwing crispa should contain two partially overlapping inversions,[6] then a crossing-over[7] within inverted regions would produce a deficiency[8] which may readily show up as a dominant character and which almost certainly would be lethal when homozygous. The frequency with which such cross-overs would occur would be determined by various factors. It is probable that it would be low and that it could readily fulfill the two per cent. requirement. The crispa case, therefore, if included among unstable gene cases at all, should be included with reservations.

Changes in unstable genes usually go in one direction only, from a recessive into a dominant allel. This dominant allel is ordinarily as stable as any stable gene may be expected to be. Whether or not the change is reversible would be shown by the pattern formed by mosaic tissue. A reversible change in the case of a color character, for example, would give a double pattern, changes from light to dark showing as dark spots on the light background and changes in the opposite direction showing as light spots upon dark spots. Only one case of this type has been described so far (2). In several instances it has been claimed that gene changes occur in both directions (62, 48) but a careful analysis of the evidence presented makes these claims at least questionable. A close approach to a reversible change was described by Emerson (31) who observed that some of the solid red cobs of maize originating from variegated seeds had a few variegated seeds. That, however, was not a general characteristic of red cobs but was limited to a certain strain of maize. A good case of a reversible change has been described by Anderson and Ter Louw (2) in mosaic pericarp of

[6] An *inversion* is a reversal of the linear sequence of genes in one segment of a chromosome relative to an adjoining segment (Darlington).—Editors.

[7] *Crossing-over* is an interchange of corresponding segments of homologous chromosomes.—Editors.

[8] A *deficiency* is the loss of a segment of a chromosome.—Editors.

maize where the pattern produced indicates that the changes occur in both directions, from white to red and from red to white.

It is not easy to determine whether or not an unstable gene changes into its stable recessive allel because of great variability in frequency with which changes in unstable genes usually occur. To distinguish a constant allel from one changing with low frequency, a very large number of observations would be essential. Evidence available at present indicates that if such changes occur at all they occur with much lower frequency than changes to the dominant allel.

In addition to changes from the unstable recessive to the stable dominant, another type of change is evident among unstable genes, *viz.*, changes to various allels differing from each other in the rate with which they change. It is probable that these changes are fairly common although they are not easy to detect because other factors cause rate of change to vary. The best evidence of changes from one unstable allel into another is available in the case of the unstable miniature-3 gene of *Drosophila virilis* (19, 21). Three miniature allels are known in the miniature-3 series, *viz.*, miniature-alpha which is unstable both in germinal and in somatic tissues, miniature-gamma which is unstable in somatic tissues only and miniature-beta which is stable as far as changes into the wild-type are concerned. Miniature-alpha and gamma revert frequently into the wild-type and in addition to this, alpha rarely changes into gamma or beta, gamma rarely changes into alpha or beta, and beta rarely changes into alpha or gamma. Similar changes from one unstable allel into another were observed in maize (31) where pericarp variegation of the calico type, which affects somatic tissue and germ-cells, changes into dark crown variegation affecting somatic tissues only; in *Pharbitis* (44) where the unstable duskish gene produces several unstable allels; and in *Polystichum* (4) where about six unstable allels for chlorophyll variegation are known. Rate of change from one unstable allel into another is low when compared with the rate with which unstable allels change into wild-type.

Effect of various factors on rate of change in unstable genes. It is known that the rate with which unstable genes change into the wild-type is influenced by various internal factors. Outstanding among these are various modifying genes. Three genes are

known, for example, which greatly increase rate of change in somatic cells of miniature-3 alpha and gamma of *Drosophila virilis* (22) and one gene is known which increases that rate in the germ-cells of miniature-3 alpha (23). Emerson (32) showed that modifying genes are responsible for increase of mutability of variegated pericarp of maize in crosses, *viz.*, when the unstable gene is in a heterozygous condition. A similar condition is probably responsible for such an increase in mutability as observed in *Mirabilis* (13). A decrease in rate of change of the globifera gene of *Antirrhinum* (41) was observed when this gene was heterozygous with its chlorantha allel and when it was compared with the rate of change observed in homozygous globifera or in the globifera/nicotianoides combination. Kihara (47) described a similar occurrence in *Celosia* where instability of the gene for the anthocyan color was decreased when it was combined with its stable allel.

Rate of change of certain unstable genes is affected by various ontogenetic factors. Evidence indicates that in *Delphinium* the unstable rose gene reverts to purple with constant frequency throughout all stages of ontogeny, but the unstable lavender gene reverts with high frequency in the early embryo and in late stages of flower development and it is either constant or reverts with low frequency at other stages of ontogeny (24). Anderson and Eyster (1) found that rate of change in variegated pericarp of maize increases toward the end of development of seeds. In *Pharbitis* Imai (44) found that yellow-inconstant-1 and flecked revert with a high rate early and late in ontogeny and with a low rate at other stages, while yellow-inconstant-2 reverts with low frequency at late stages and with high frequency at other times. As already mentioned, miniature-alpha of *Drosophila virilis* (21) is unstable both in somatic and in germinal tissues and miniature-gamma is unstable only in somatic cells. As far as is known at present, the reddish-alpha of *virilis* has the most restricted period of instability, reverting only at a maturation division of heterozygous females (20).

Several other factors affect rate of change of unstable genes. It is known that age reduces frequency of reversions in reddish-alpha of *Drosophila virilis* (20). It has been observed also that sex affects rate of change of the miniature-gamma gene, the rate in males being about twice as high as in females (26).

From the previous discussion it is evident that unstable genes are sensitive to various internal factors. Very few tests have been made to determine the effect of external factors on these genes. Eyster (34) reported in variegated pericarp of maize a significant difference in rate of change between material grown in a warm and that grown in a cool climate, the cool conditions producing an increased rate. Demerec (25, 28), however, found that neither a ten degree centigrade difference in temperature nor x-ray radiation significantly affected rate of change of the unstable miniature-3 gene of *Drosophila virilis*.

Characters determined by unstable genes. All types of characteristics are represented among those which are determined by unstable genes. As may be expected, characters showing color variegations are most frequently observed since they are easily detected. Many of them have also been preserved by breeders of ornamental plants and thus made available for study.

The variety of characteristics determined by unstable genes is best illustrated in the following list which is not intended to be complete. Various plant and flower color variegations are described in *Antirrhinum* (64, 5), *Celosia* (62), *Dahlia* (50), *Delphinium* (64, 24), *Hordeum* (58), *Impatiens* (39, 46), *Lathyrus,* (55), *Mirabilis* (14), *Miosotis* (10), *Nigella* (63) and *Zea* (30, 40); chlorophyll variegations are described in *Antirrhinum* (5, 49), *Barbarea* (3), *Capsella* (15), ferns (4), *Mirabilis* (14), *Nigella* (63), *Pisum* (38), *Plantago* (42) and *Viola* (12); of the morphological characters a description was made of globifera in *Antirrhinum* having small petals and only female flowers (5); phantastica and graminifolia in *Antirrhinum,* both having narrow leaves (6, 56); large grains in *Oryza* (61); contorta with whirled leaves in *Plantago* (42); ramosa with branched inflorescence in *Plantago* (64); dwarf plants in *Portulaca* (8); and of the physiological characters a sterility factor was described in *Antirrhinum* (5) and several such factors in *Oryza* (60, 52, 48). In *Petunia* an unstable gene was described which affects both color and size of flowers at the same time (57). In *Pharbitis* seventeen unstable genes are known affecting flower color, chlorophyll and various morphological characteristics (44). In *Drosophila virilis* three unstable characters are known, one affecting color of the body, one color of eyes and another size of wings (16, 17, 18).

Occurrence of unstable genes in various organisms. According to my list, there are at least 63 cases of unstable genes described among plants. In some plants numerous cases have been described; as many as 17 in *Pharbitis* and 7 in *Antirrhinum;* in others, relatively few are known as in maize where only two cases are known, one of which is still unpublished. This suggests that unstable genes may occur with different frequencies in different plants. As, however, there are many factors which can influence detection of an unstable gene, this difference is by no means conclusive.

Among animals only a few cases of unstable genes have been described. Three cases in *Drosophila virilis* (16, 17, 18) are unquestionably of that type. In *Drosophila melanogaster,* however, which is genetically much better known than *virilis,* only one character has been described as determined by an unstable gene (54). Patterson (53) reported a miniature wing character of *melanogaster* as being unstable, but the character was lost before it was fully investigated. Since at least ten times as many mutations have been observed in *melanogaster* as in *virilis,* difference in the number of unstable genes found in the two species may be due to specific differences between them.

As a possible case of an unstable gene, Castle (9) described repeated occurrence of a mosaic pattern in a strain of rabbits. Ferwerda (37) also described an eye character in *Tenebrio* which frequently gives spotted eyed and normal eyed individuals and which may be due to instability of the gene.

Hypothesis. DeVries (64) was the first to describe breeding experiments involving unstable genes. He recognized both somatic and germinal mutations and called them atavisms, *i.e.,* reversions to an ancestral form. Correns (14) realized that in homozygous variegation changes occur from a homozygous to a heterozygous condition. He visualized this change as due to the presence of an inactive gene for self-color among a gene or genes for variegation, which inactive gene becomes, under certain conditions, active, thus producing a change in the genotype.[3]

Emerson (30) was the first to interpret the behavior of variegations as being due to changes in the genes. For variegated pericarp of maize he assumed that red patches are the result of changes of the gene for variegation into its red allel. This interpretation

is generally accepted today. It agrees with all known facts. Recently, however, the cause of several variegated characteristics (51, 11) was traced to abnormal chromosome behavior and the suggestion was made (59) that the behavior of unstable genes might be interpreted in a similar manner, *i.e.,* they may be small duplications[9] and the appearance of the new form may be due, not to a change in a gene, but to a segregation of genes already present. The main opposition to such an interpretation lies in the fact that in all cases attributed to unstable genes, the change goes from the recessive to the dominant allel. If both recessive and dominant allels were present in the unstable form, an improbable assumption would be essential, namely, that a dominant allel in combination with two recessives does not show its dominant effect. The analysis made with unstable genes of *Drosophila virilis* (17, 18, 20), where behavior of chromosomes was followed through a number of markers, indicates that no gross chromosomal abnormality is responsible for the changes observed. In case of reddish the cause for changes was traced down to within a region of only 2.8 cross-over units,[10] in which region it is known that the reddish gene is located. At present, Emerson's explanation is by far the most probable. A positive test on whether or not unstable genes are connected with duplications is now possible for *Drosophila* characters through use of the salivary chromosome method. These tests will be made as soon as the salivary chromosome structure of *virilis* is better known.

Correns (15), describing chlorophyll variegation of *Capsella,* again considers the theory of variegation. He looks upon changes as too frequent to be called mutations. He thinks of them rather as a disease of the gene, the varying degrees of disease being expressed as lighter or darker variegation. According to Correns, a gene which determines variegation may be pictured as a large molecule which consists of many identical atoms. The number of atoms is variable; it can increase or decrease. To each number of atoms in the molecule, corresponds a definite ratio of green to white tissue on the plant. Change in number of atoms can occur at any cell division. Only two stages are constant, *viz.,* the form

[9] A *duplication* is the occurrence of one segment of a chromosome twice in the same complement. (Darlington).—Editors.

[10] A *cross-over unit* is a one per-cent frequency of interchange between a pair of linked genes.—Editors.

with the maximum number and the one with the minimum number. The first combination determines green and the second white cells in case of a chlorophyll variegation. It is evident that Correns considered variegations as caused by changes in the genes although he did not call these changes mutations.

At the Toronto meeting of the American Association for the Advancement of Science (1920) E. G. Anderson suggested that unstable genes are composed of two kinds of particles, one responsible for the recessive type and one for the dominant type, and that changes observed in these genes are due to assortment of these particles within a gene. The gene responsible for variegated pericarp of maize, for example, would be composed of two kinds of particles, one kind determining red color and the other white color. Whenever the number of red particles is in excess of the threshhold, color of the cell would be red, and when it is below the threshhold, color would be white. The gene for stable red would have red particles only and the gene for stable white, white particles only. This suggestion has been elaborated and developed as an hypothesis by Eyster (33, 35, 36) who gave the name *genomeres* to particles which are supposed to be independent components of a gene. The first impression given by the genomere hypothesis is that it is simple and can explain not only the behavior of unstable genes but also the behavior of other genes as intimated by Eyster. When a close analysis of various cases is made, however, it becomes evident that in its simple form the hypothesis is applicable in only very few instances. To explain other cases, additional assumptions are essential, and if the whole field is to be covered, these assumptions become so numerous and involved as to make the whole hypothesis highly improbable. If it is assumed that an unstable gene is composed of two kinds of genomeres, that they divide at each gene division and segregate at random to daughter genes, it can be estimated from formulae developed by Dr. Sewall Wright (unpublished) that to obtain a constant rate of change of 7^{-5} per cell generation observed in unstable rose of *Delphinium* (24), about 4000 genomeres would be required, but that with such a high number of components thousands of cell generations would be needed before the constant rate of change is approached. The observed behavior of the rose gene, therefore, cannot be explained by the genomere hypothesis

except, probably, by making involved assumptions. Similarly, observed facts that certain genes are stable at one stage of ontogeny and unstable at another, that certain genes change with different rates at various stages of ontogeny, and that various factors may influence rate of change can be explained by the genomere hypothesis only with the use of additional assumptions. If unstable genes were composed of genomeres, that should be true also of stable genes. In that case, it would be reasonable to assume that a mutation would begin with a change in a genomere, which process would be expected to produce unstable genes with high frequency. Ample evidence is available to show that this is not the case.

It has been suggested by Demerec (19, 24) that changes in unstable genes are caused by chemical processes rather than by mechanical assortment of particles within these genes. According to this view, an unstable gene is a chemically unstable entity changing into another definite form. Chemical reactions responsible for these changes may be influenced by various conditions of the gene environment. Thus, rate of change may vary at different stages of ontogeny, in different sexes and it may be influenced by certain other genes. It has been pointed out (27) that changes in unstable genes and changes in stable ones are chemical processes similar in nature, and also that there is no clear-cut difference between stable and unstable genes. Certain genes which are now called stable would be included in the unstable group if they were changing with the same rate in somatic cells where these changes could be easily detected, as they are changing in the germ-cells, where they are not easy to detect. At the same time a working hypothesis has been outlined, picturing a gene as a complex organic molecule and changes in genes as either slight rearrangements or changes in a molecular group of that molecule. An unstable gene, according to this concept, would have a molecular group in a chemically labile condition. Negative results obtained in temperature and x-ray treatments of unstable genes (25, 28) are interpreted as being due to the fact that the effects of these two treatments are so slight, in comparison with the natural rate of changes, that it is not possible to detect them.

Observations made on stable genes indicate that about seventy per cent. of the changes among them eliminate the gene (29). This elimination process is pictured as due to loss of reproductive

power of the gene because of the change which occurs. If changes in genes are considered as chemical reactions, that evidence would indicate that a gene may stand slight changes only; any extensive change destroys its power to reproduce and thus eliminates the gene.

The present day genetics concept visualizes the appearance of an organism as a result of an interaction of the whole set of genes the organism possesses and the environment in which it develops. A change in any of the genes, called a mutation, is liable to upset the balance of that system and show up on the organism as a character, usually as an abnormality, in some respect poorer than the wild-type. The appearance of a characteristic may be due to a change in a certain gene. That does not mean, however, that any single gene is entirely responsible for the development either of a particular character or a particular organ. Ample evidence is available to show that the final effect on the organism is produced through the interaction of the whole complement of genes, although certain of them may have greater influence on the expression of certain characteristics than others.

Studies with deficiencies, *viz.,* material where one or several genes are missing, show that the majority of deficiencies are lethal to the organism when present in a homozygous condition. This suggests that the presence of at least the majority of genes is essential in order that an organism may live. Moreover, the work with *Drosophila melanogaster* deficiencies (29) indicates that many of them are cell-lethal, *viz.,* that even a small group of cells located among normal tissues but containing a homozygous deficiency cannot exist. This suggests that genes are active in every cell and that, probably, the majority of them perform there a function highly important in the vital processes of the cell.

It has been pointed out (29) that a change in a gene which produces a visible effect (mutation), since it is detrimental to the organism, is not an effective means of evolution unless, if due to the change in the environment, the detrimental effect is either eliminated or changed into a beneficial effect. It is likely that more important for evolutionary processes are changes in genes which have a negligible effect on the organism. Accumulation of such changes in isolated groups of species may result in a significant difference between these groups. An addition of a new gene

(locus) which eventually would become so important as to act as a cell-lethal if deficient, would produce a fair degree of sterility between a group possessing such a locus and another one not having it and would, therefore, constitute an important step toward the formation of a new species.

It seems unnecessary in this review to discuss the well substantiated fact that genes are located in chromosomes, that they are there in a definite order which has a high degree of constancy. It may be mentioned only that it is not definitely known how much the appearance of a chromosome is determined by the genes it carries. There is evidence which indicates that there are chromosomes and chromosomal regions which carry very few, if any, genes but which in appearance and behavior are almost indistinguishable from the chromosomal regions containing genes. On the other hand, there is reason to believe that genes are even present in low organisms in which chromosomes are not detectable, and if that is true, the genes in these cases are independent of chromosomes. It seems justified to look upon a gene as a structure which was originally independent of a chromosome, but which, in early stages of evolutionary progress, either initiated formation of a chromosome by several of them forming a gene string or became associated with structures which later evolved into chromosomes.

In concluding this review, it may be pointed out that our knowledge about the gene is still in its infancy. Experimental methods (x-rays and salivary chromosomes) which became available to geneticists recently offer a means for more direct attack on the gene problem than was possible only a short time ago and give hopes for faster progress in the near future.

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RESPIRATION

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Introduction

By the term respiration is now understood the complex processes taking place in every living cell which involve a release of chemical energy utilisable in part for the building up of compounds of higher energy content and for other vital processes needing a supply of energy. While this release of energy is effected among bacteria in a variety of ways, in the vast majority of plants the normal respiratory process involves oxidation of carbohydrates and fats into carbon dioxide and water, oxygen being absorbed from the external medium to effect the oxidation, whence the whole complex of processes is described by the term "aerobic respiration." As is well known, a breakdown of carbohydrates takes place in plants when oxygen is excluded and a type of respiration, now generally known as "anaerobic," then occurs in which carbon dioxide is produced without a corresponding absorption of oxygen.

Investigations on respiration during the last five years have been concerned with both aerobic and anaerobic respiration in a variety of plant species and different plant organs in various stages of development. For most of these researches methods of measuring respiration have been employed which have for long been proved of service. These methods generally used involve determination of rate of evolution of carbon dioxide by the respiring tissue, the principle generally involved being the absorption in a standard solution of alkali of the carbon dioxide evolved. The solution is then titrated against standard acid, the decrease in alkalinity of the solution giving a measure of the amount of carbon dioxide absorbed. This method, in some modification of the form devised many years ago by Pettenkofer, is still regarded as the most reliable and most suitable for measurement of the respiratory activity of much plant material. An interesting modification of this method has been described by P. Emerson (11). The carbon dioxide is absorbed by barium hydroxide solution as in the usual procedure, but instead of titrating this with standard acid the amount of barium carbonate precipitated is determined by measur-

ing the height of the column of this precipitate in a tube under standard conditions.

The Pettenkofer method and its usual variants require a comparatively large amount of respiring material and experiments often have to be carried out over a few, and not infrequently, many hours, in order that the amount of carbon dioxide evolved and then absorbed by the alkaline solution shall be sufficient to alter the strength of the solution by a significant amount. Hence, where the respiration of small quantities of material over small intervals of time is to be measured the Pettenkofer method is inapplicable. For such work manometric methods, such as that involving the use of the Barcroft differential manometer and its variants, are frequently employed. Thus, in an investigation on the respiration of strawberry fruits, to which reference will be made later, A. R. Gerhart (16) used the Krajnik type of manometer. The principle involved is to allow the material to respire in a confined space and to absorb the carbon dioxide evolved with alkali. The gas in the space then loses the oxygen absorbed by the tissue and gains nothing from the latter. Consequently the change in volume under constant pressure is the volume of oxygen absorbed by the tissue. Obviously the smaller the respiration chamber the more sensitive the apparatus, so that the absorption of quite small amounts of oxygen can be determined.

But as the Pettenkofer method only gives a measure of carbon dioxide evolved, so the manometric methods only give a value for the oxygen absorbed. It is true that at the end of the experimental period with the manometric method the amount of carbon dioxide evolved by the alkali can be determined, but a series of observations on one sample of material can be made on oxygen intake only.

To overcome these limitations in respiration measurements inherent in the usual methods, W. Stiles and W. Leach (37) have developed the instrument known as the katharometer for the measurement of small amounts of respiration. The principles involved in the measurement of respiration by this instrument are as follows. When an electric current is passed through a wire it becomes heated, the temperature it acquires being in part determined by the thermal conductivity of the medium surrounding the wire, since the rate of removal of heat from the wire depends

on the thermal conductivity of the surroundings. Now the thermal conductivities of various gases are different, so that if the heated wire is contained in a vessel in which material is respiring, the replacement of oxygen by carbon dioxide will result in a change in the thermal conductivity of the gas in the vessel and hence in the temperature and electrical resistance of the wire. The replacement of air or oxygen by carbon dioxide has a very significant effect on the thermal conductivity of the gas and in the katharometer method of measuring respiration the output of carbon dioxide by respiring tissue is followed by determining the changes in electrical resistance of a heated wire in the respiration chamber. This method can be made very sensitive; indeed, by its means a change in carbon dioxide concentration of no more than .001 per cent. of the volume of the respiration chamber can be measured. Various improvements in the practice of the method were subsequently described by W. Leach (26). Thus by clock-work and photographic devices the apparatus can be made self-registering, while by the attachment of a manometer to the plant chamber changes in pressure can be measured and so data obtained for calculating not only the carbon dioxide evolved but the oxygen absorbed. Further, in this paper Leach also describes a device, based on the same principle as that used in the katharometer, by which changes in pressure can also be registered automatically. It is, however, an important limitation of the katharometer method that it is applicable in its present form only when the oxygen and carbon dioxide concentrations in the respiration chamber are changing. If the concentration of some other gas in the medium is changing as well, as, for example, if ethyl alcohol vapour should be given off by the respiring tissue, changes in thermal conductivity result from changes in both carbon dioxide and ethyl alcohol concentration. But if such a complication is not present the katharometer method affords a way of obtaining a continuous record of both carbon dioxide output and oxygen intake by small quantities of respiring material such as single germinating seeds, isolated small leaves and individual moss plants.

As regards the measurement of respiration, reference should be made to a paper by J. J. Willaman and W. R. Brown (47). These workers have devised a method for determining the amount of carbon dioxide dissolved in the sap of twigs and have applied

the method to work on apple twigs. The twigs are submerged in 95 per cent. alcohol in a closed vessel, the gas then removed under reduced pressure and the carbon dioxide absorbed in standard alkali and determined by subsequent titration of the alkali. The amount of carbon dioxide which can be removed from the twigs in this way is not inconsiderable, namely, about 150 to 250 mg. per kilo. of twigs at 0°, the amount declining with increase in temperature. Willaman and Brown consider that the presence of this dissolved carbon dioxide may explain three apparent phenomena of plant respiration. In the first place, when apple twigs are transferred from a lower to a higher temperature there results an increase in respiration which reaches a maximum and then falls subsequently to a level rate, the temporary rise being greater the lower the initial temperature. This temporary rise in respiration rate to a height above that characteristic of the temperature is attributed to lower solubility of carbon dioxide in the cell sap at the higher temperature with consequent release of the excess gas. Secondly, if tissue is kept in a closed vessel in which respiratory carbon dioxide accumulates and this carbon dioxide is removed and the respiration rate then measured, this rate also rises to a maximum and then falls to a level rate. The temporary rise is probably accounted for in part by release of carbon dioxide dissolved in the cell sap, since its concentration in the sap will be higher when there is a higher partial pressure of the gas outside the tissue than when the partial pressure is low. It is also suggested that higher acidity of the sap resulting from accumulation of carbon dioxide in it may also lead to a higher rate of carbon dioxide production. A third phenomenon connected with the solution of carbon dioxide in sap is the lower rate of carbon dioxide output by varieties of apples which possess greater winter hardiness. These varieties contain the lowest concentration of carbon dioxide in the sap but the cause of these relationships is not understood. In any case it may often be necessary, as Willaman and Brown point out, to distinguish between the measured rate of carbon dioxide output and the rate of carbon dioxide production by living tissue.

From some observations on the rate of respiration of potato tubers of different sizes in the case of three varieties, W. H. Michaels (32) concluded that the lenticels are the chief channels

of gaseous exchange between internal tissues and the surrounding air. While there is every reason to believe that this is so, it is difficult to understand how the data presented justify this conclusion, but the data themselves are not without interest. Thus, in the variety Early Ohio the respiration rates of large, medium and small tubers were found, respectively, to be .0296, .0312 and .0329 mg. carbon dioxide per sq. cm. of surface per hour, the corresponding respiration rates per lenticel being .0126, .0123 and .0075 mg. carbon dioxide per hour. The decrease in the rate of carbon dioxide production per lenticel in small tubers as compared with large ones is attributed to greater frequency of lenticels in the small tubers.

The Influence of External Conditions on Respiratory Activity

The chief external factors which might normally influence intensity of respiration in plant material are temperature, light, and the oxygen and carbon dioxide present in the atmosphere surrounding the tissue. A number of observations have been made during recent years also on the effect on respiration of various other substances when introduced into the external medium.

It is well known that the general effect of temperature on respiration over the range at which growth of most plants takes place, say from 5° to 30° C., is that the rate of respiration is increased from about twice to 2.5 times for a rise of 10° C. A number of recent researches, while indicating minor differences, have mainly served to extend the list of materials which obey the rule. Thus A. R. Gerhart (16) found that between 5° and 25° intensity of respiration of strawberry fruits is increased by almost exactly 2.5 times for a rise of 10°. Above 25° C., however, although the initial rate of respiration increases with increase in temperature up to 36.5° C., the initial rate is not maintained, the rate of respiration falling off more or less rapidly. The well-known "time factor" of F. F. Blackman is thus operative here. Gerhart's own opinion is that this time factor results from inability of oxygen to penetrate the cells rapidly enough to maintain the higher respiration rates. This further results in a certain amount of anaerobic respiration with the production of ethyl alcohol and other toxic substances which exert a deleterious effect on respiratory enzymes.

The same general relation between temperature and intensity of respiration was found, for the most part, by A. Hée (24) in seedlings of *Vicia faba, Zea mais* and *Lupinus,* in various leaves and in bulbs of *Allium,* as well as in the fungus *Sterigmatocystis nigra.* Similar results were obtained with seedlings of *Phaseolus aureus* by W. J. Crozier and A. E. Navez (9) and with seedlings of *Zea mais* and *Lupinus albus* by Pei-Sung Tang (40, 41, 42, 43). F. Kidd and C. West (25) found a similar effect of temperature on respiration in senescent apples. An interesting finding by Pei-Sung Tang is that oxygen absorption and carbon dioxide output are not affected to the same extent by temperature.

W. H. Michaels (31) concluded that change in temperature of the root of etiolated seedlings of *Phaseolus vulgaris* acted as a stimulus to the respiration intensity of the shoot. But fluctuations from hour to hour in respiration rate of the same root as measured by Michaels are so great that some doubt must be felt for the justification of the conclusion drawn. For example, respiration rate of roots grown at 15° C. during a number of successive hours varied from .77 to 9.48. Obviously any increase in respiration rate to be really significant would have to be very large, certainly much larger than those recorded which are of the same order as the fluctuations in respiration rate under constant temperature conditions.

After temperature, oxygen concentration as an external factor in respiration has attracted most attention during recent years. Until about 1930 the view was generally held that oxygen concentration was without effect on intensity of respiration unless the former was so low that anaerobic conditions were approached. However, in 1930, F. F. Blackman (7) announced that with apples in an atmosphere of oxygen and nitrogen, rate of respiration falls with increasing oxygen concentration until a minimum rate is reached in the neighbourhood of about 5 to 9 per cent. oxygen. Above this concentration the rate of respiration increases with increase in oxygen concentration until 100 per cent. oxygen is reached. In the same year W. B. Mack (28) published results of considerable research on the relation of temperature and oxygen concentration to respiration and growth of young wheat seedlings, respiration of these being measured at five different temperatures between 10° and 30° C., inclusive, and in twelve different oxygen concentrations ranging from .6 to 98.3 per cent. Here also the

relation of respiration rate to oxygen concentration was found not to be simple. For each temperature it was found that with increasing oxygen concentration from the lowest concentration used, the intensity of respiration also increased until a maximum was reached, the actual oxygen concentration at which this occurred depending on the temperature. Thus at 10° it occurred at from 6.3 to 9.8 per cent. and at 30° at from 10 to 16 per cent. With further increase in oxygen concentration there resulted a fall in respiration rate until a maximum was reached after which, with further increase in oxygen concentration, the rate again rose until a second maximum occurred at 90 or 95 per cent. oxygen. The rate of respiration at the highest oxygen concentration used, 98.3 per cent., was always lower than at 90 per cent. It seems clear that further investigation of the relation between respiration intensity and oxygen concentration is desirable.

The effect on respiration of various non-essential substances has been examined by various workers during the last five years and special attention has been directed to the effect of two toxic substances, hydrocyanic acid and ethylene. As regards the former, A. C. Shill (36) examined the effect on respiration of *Citrus* plants of a dosage of hydrocyanic acid about equal to that used in fumigation. It was found that the treatment brought about initially an increase in respiratory activity amounting to about 75 per cent. but that after about 35 hours the respiration rate returned to normal. An important contribution on the effect of hydrocyanic acid on respiration of potato tubers was published at about the same time by C. S. Hanes and J. Barker (22). They also found that with this material respiration rate at 15° C. increases as a result of exposure to an atmosphere containing hydrocyanic acid (.14 cc. to 1.45 cc. per litre) and then falls more slowly. In the highest concentration employed, however, the tissue suffers injury, but in the lower concentrations this is not the case. So long as the tissue is not injured the respiratory quotient, CO_2 evolved : O_2 absorbed, remains constant at about unity but in the high concentration the quotient rises to about 1.2.

The rise and fall in respiration rate accompany a corresponding rise and fall in sugar content of the tuber, and in moderate concentrations of cyanide it is thought that changes in respiration rate are due to corresponding changes in sugar concentration. These latter are themselves thought to be due to the direct effect of the

hydrocyanic acid on the starch-sugar relationships in the tuber. In the highest concentration of cyanide, when the respiration rate is falling from its maximum value, the ratio of respiration rate to sugar concentration falls, an effect attributed to inactivation of respiratory enzymes.

The effect of ethylene on respiration of wheat seedlings has been investigated by W. B. Mack and B. E. Livingston (29). A series of experiments was carried out similar to those described by the first named author to which reference has already been made, but with the addition of .1 per cent. ethylene to the experimental atmospheres. Various combinations of temperature and oxygen concentrations were employed as in the experiments without ethylene. It was found that this substance in the concentration employed has practically no effect on respiration at all temperatures when oxygen concentration is low (.6 per cent.) or high (75 per cent.; the highest concentration used in the experiments with ethylene). With oxygen concentrations of 20 and 50 per cent. also ethylene had little effect on respiration or retarded it somewhat, while in atmospheres containing 10 per cent. and 30 per cent. oxygen, ethylene accelerated carbon dioxide production. The complexity of the results very definitely indicates need for further investigation.

An increase in respiration rate of about 7.5 per cent. was observed by J. Green and A. H. Johnson (17) in bean leaves as a result of spraying with crude petroleum oils containing more than 16 per cent. sulphonatable residues, whereas the more refined oils brought about a decrease in respiration rate. The sulphonatable residue consists largely of unsaturated hydrocarbons and of sulphur, nitrogen and aromatic compounds. An increase in respiration rate of about 50 per cent. was also observed by M. P. Masure (30) when etiolated pea seedlings were irradiated with ultraviolet radiation.

The Effect of Respiratory Substrate on Respiratory Activity

The relation between concentration of sugar in respiring tissue and intensity of respiration has already been mentioned in dealing with the work of Hanes and Barker on effect of hydrocyanic acid on respiration. In two papers Barker (2, 3) has dealt with the

general question of relation between respiration of potatoes and concentration of sugars. The investigator found the problem complicated by a temperature effect which consists of a depression of respiratory activity as a result of exposure to low temperature. The depressing effect lasts for several weeks after a return to a higher temperature. It is regarded as the resultant of two processes, both affected by temperature but in opposite directions. These processes are the accumulation of the inhibitor, which is greater the lower the temperature, and the development of the inhibitory effect which is greater the higher the temperature and which has, indeed, a high temperature coefficient. Where the depressing effect is absent, sugar content has considerable effect on rate of respiration, as already indicated by the work of Hanes and Barker.

Variations in Respiratory Activity During Development

Change in respiration intensity of plants and plant organs in different stages of development has been recently investigated in a number of cases, particularly during germination and seedling development and during development and senescence of fruits.

Reference has already been made to Mack's observations (28) on respiration of wheat seedlings. Working with seedlings initially two days old he found over a period of about another two days a continuous rise in carbon dioxide output. In an investigation on the course of respiration of germinating seeds and seedlings of sweet pea (*Lathyrus odoratus*) Stiles and Leach (38) found the course of respiration depended largely on whether the seed coats were present or removed. If the seed coat is present and intact the course of respiration shows a series of phases. First, there is a fairly rapid increase in respiratory activity as the seeds absorb water. This is followed by the second phase, a period at which respiration rate remains constant, a state of affairs terminated by rupture of the seed coat which is followed by the third phase, a very rapid rise in respiration rate to a constant value characteristic of the fourth phase. This is finally succeeded by a decline in respiratory activity. If the testas are removed, the first phase of constant respiratory activity is eliminated and the respiration rate continually increases to the maximum of the fourth phase. The constant respiration rate of the second phase thus appears to be

related to the presence of the seed coat and may be conditioned by this latter limiting the diffusion of gases to and away from the respiring tissue, or may be related to inability of the seedling to grow in a confined space. The final falling off in respiratory activity appears to be due, either wholly or in part, to the conditions of experimentation, for the seedling is maintained in a closed chamber and reduction in transpiration may effect transference of respirable material from the cotyledons to the growing points, and so to a limitation of substrate at the places where respiration is most active.

A number of investigations of this kind have been carried out with fruit. Changes in respiratory activity of apples during senescence at different temperatures (2.5°, 10°, 22.5°) have been examined by F. Kidd and C. West (25). The course of respiration was similar at the three different temperatures, the respiratory activity rising to a maximum and then falling, the maximum value reached being about 1.5 times the initial value. This peak value is reached sooner the higher the temperature. Available evidence with regard to concentration of sugars in the tissues indicates that these changes in respiratory activity cannot be explained as due to changes in concentration of the sugar substrate of respiration, and it is suggested that the observed changes are related to some change of state in the colloidal matrix of the protoplasm. Such change might lead to a greater or less amount of effective enzymes or to a greater *effective* concentration of substrate, either by elution or by increase in permeability of the plasmatic membrane between protoplasm and vacuole.

The observations of Kidd and West were made on the English variety of apple, Bramley's Seedling. According to P. L. Harding (23), who also followed the respiratory activity of apples during storage at four temperatures (−1°, +2°, 10°, 15.5°), the respiration rate at each temperature increased during development and maturity. He worked, however, with the Grimes variety.

A paper on respiration of strawberries by A. R. Gerhart (16) has already been mentioned. E. L. Overholser, M. B. Hardy and H. D. Locklin (34) have also made a study of the respiration of this fruit. They also find that respiration is more rapid in mature than in immature fruits. Other aspects of the respiration of fruits will be dealt with later.

The Respiratory Quotient

It has for long been realised that a certain amount of insight into the respiratory process might be obtained from an examination of the relation between the amount of carbon dioxide evolved in respiration to the oxygen absorbed. The ratio of these two quantities is the respiratory quotient or respiratory coefficient, frequently now designated by the symbol R.Q. In particular a knowledge of its value might assist in deciding what materials were actually utilised as respiratory substrate. We have already noticed that in potato tubers concentration of sugar appears to have a definite determining effect on the rate of respiration, and if only sugar or some other carbohydrate is utilised, and if this is oxidised completely to carbon dioxide and water, and if there is no internal source of oxygen, and if all the carbon dioxide produced escapes from the tissues, then the respiratory quotient should be unity. If, on the other hand, fat is utilised, the respiratory quotient should be considerably below unity, actually in the neighbourhood of .7, since fats contain many fewer oxygen atoms than carbon atoms so that for the complete oxidation of a fat much more oxygen has to be absorbed relative to the amount of carbon in the substrate than for the complete oxidation of a sugar substrate.

Germinating seeds provide excellent material for demonstrating the influence of substrate on the respiratory quotient. Thus Stiles and Leach (39) examined the change in value of the respiratory quotient during germination of seeds of a number of different species with different food reserves. For each species the quotient exhibits a continuous and characteristic change in value during germination. In *Zea mais,* where the reserve is chiefly starch but where a little fat is present, the quotient is initially about unity, falls to a minimum value of about .75 and then rises slowly towards unity. These changes in value of the quotient may be explained by supposing that at the beginning of germination a small quantity of sugar is present in the seed and this is at once used for respiration. As germination proceeds, fat becomes utilised and is either completely oxidised to carbon dioxide and water direct or, perhaps more probably, is first oxidised to sugar. In either case this would lead to a lowering of the respiratory quotient as actually observed. As the fat is removed, sugar derived from hydrolysis of starch by diastatic enzymes becomes the principal substrate and the respi-

ratory quotient therefore rises towards unity. Essentially similar behaviour was observed with other seeds in which the reserve material is chiefly starch, namely, *Lathyrus odoratus, Vicia faba* and *Pisum sativum.* In buckwheat (*Fagopyrum esculentum*) the initial fall in value of the respiratory quotient was not observed; it is either not present or is extremely rapid and the small quantity of fat in this seed appears to be utilised very rapidly for the quotient rises from its initial low value to near unity in a very few days.

In seeds containing fats as the chief reserve there are minor irregularities, but, as is to be expected, the respiratory quotients are below unity. In *Ricinus communis* the respiratory quotient of germinating seeds falls regularly from about .85 to .5 in about 5 days, an observation in agreement with determinations made by A. I. Ermakoff and N. N. Iwanoff (12) and by J. R. Murlin (33). The latter even obtained values of the respiratory quotient of seedlings (with seed attached) as low as about .3. These low values can be readily explained only on the view that the fat is converted to sugar at a much greater rate than it, or sugar derived from it, is respired. With other fat-containing seeds, *Helianthus annuus* and *Cucurbita pepo,* there is generally first a rise in the quotient from about .75 to a maximum somewhat higher, after which there is a regular fall. The transitory rise may be due to utilisation of a little carbohydrate present in the seed along with the fat.

As shown by Aubert more than 40 years ago, the peculiar metabolism of succulent plants results in respiratory quotients in these plants of very varying magnitudes. The metabolism of succulents is still far from understood and in recent years fresh attacks have been made on the problems involved. T. A. Bennet-Clark (5), with a new type of apparatus (4), has examined the gaseous exchange in detached leaves of *Sedum praealtum.* When maintained in the dark the rate of carbon dioxide output falls, reaching a minimum about 6 hours after darkening; it then rises to a value which remains approximately constant for many days. At the same time the rate of oxygen intake rises to a maximum value, then falls to a minimum and then again rises until the rate of oxygen intake exceeds that of carbon dioxide output. As a result, the respiratory quotient for a succession of two-hour periods shows the following values: 1.46, .55, .17, .30, .78, .86, 1.40, 1.1. It will be recalled

that the generally accepted view of metabolism of succulent plants was that, in addition to some carbohydrates undergoing complete breakdown to carbon dioxide and water, some of the sugar suffers only partial oxidation with formation of malic acid according to the equation

$$2\ C_6H_{12}O_6 + 3\ O_2 = 3\ C_4H_6O_5 + 3\ H_2O$$

Formation of a certain amount of malic acid in this way would thus involve absorption of oxygen for which no corresponding carbon dioxide is evolved and hence the measured respiratory quotient would be less than unity. Actually, when darkened, the acid content of leaves of *Sedum* rises until it reaches a maximum about 8 hours after the leaves are first darkened. For the next 8 to 12 hours the acid content falls, after which there is a further slight rise followed by a slow long-continued fall. It would appear then, that for the first 8 hours of darkness carbohydrate is being converted into malic acid and during this time the respiratory coefficient becomes considerably lower than unity. During the next period, when the acid is disappearing, the respiratory quotient rises and reaches values above unity and it is tempting to attribute this to utilisation of the malic acid as respiratory substrate. But the respiratory quotient, if the whole of the respiration took place in this way, would be 1.33, while actually still higher values were obtained; indeed, in one experiment a value as high as 2.05 was observed. The conclusion is therefore drawn that breakdown of carbohydrate is not directly through malic acid but through some other intermediate substance which requires less oxygen for its breakdown and the following scheme is suggested:

$$\begin{array}{l}
\text{Polysaccharide} \\
\quad\downarrow \\
\text{sugar} \\
\quad\downarrow \\
\text{intermediates} \longrightarrow \text{malic acid} \\
\quad\downarrow \\
CO_2
\end{array}$$

In this connection it would be interesting to know more exactly what takes place in other tissues containing much organic acid.

Unpublished observations in the reviewer's laboratory indicate that in apples, which contain much malic acid, the respiratory quotient may be well above 1.33, but there is at present no evidence that this is a general phenomenon for tissues containing much acid. In strawberries the available evidence presents contradictions for Gerhart (16) estimated the respiratory quotient to be about 1.2 while Overholser, Hardy and Locklin (34) found it to be about .9. Further observations on this question are obviously desirable and will no doubt provide valuable help towards solving the problem of the respiratory mechanism.

Anaerobic Respiration

Insight into the respiratory mechanism has also been sought by the study of respiration in absence of oxygen and particularly the quantitative relationships between aerobic and anaerobic respiration. This had already been attempted with much success by F. F. Blackman and P. Parija (6, 8, 35) for the apple and as a result of their work a scheme was evolved to account for the relationship found experimentally. In brief, according to their scheme, respiration involves several stages. These are (1) hydrolysis of reserve carbohydrate to normal hexose, (2) activation of the hexose, (3) glycolysis of the activated hexose to intermediate products, (4) respiration in a restricted sense which takes a different course in absence and in presence respectively of oxygen. In absence of oxygen the products are alcohol and carbon dioxide, in presence of oxygen part of the intermediates is completely oxidised to carbon dioxide and water while part is built back into the system by a process of oxidative anabolism.

In more recent years descriptions of further work more or less on these lines have been published dealing mainly with seedlings and fruit. As regards the former, W. Leach and K. W. Dent (27) have examined the relation between aerobic and anaerobic respiration of germinating seeds in which the principal reserves are fats, while R. H. Dastur and R. M. Desai (10) have examined certain features of aerobic and anaerobic respiration in germinating rice seeds in which the reserve is chiefly carbohydrate.

In the 'fat' seedlings, those of *Ricinus communis, Helianthus annuus* and *Cucurbita pepo,* change in the surrounding medium from air to nitrogen brings about a rapid fall in respiration fol-

lowed by a continuous slow further falling off in respiration rate. On return to air after a 24-hour period in nitrogen the respiration rate rises rapidly and finally reaches normal for the respiration of the seedling in air. Leach and Dent think the behaviour described indicates that the respiratory substrate is not fat since the former appears to become exhausted if the seedlings are kept in nitrogen. The falling off of respiratory activity in nitrogen might be attributed to inactivation of enzymes concerned in respiration, but in this case it would scarcely be expected that the seedlings would resume so rapidly their normal respiratory activity when re-transferred to air.

It may be noted that the behaviour of these seedlings, like those of rice examined by Dastur and Desai, and of other seedlings containing much starch examined by Leach in researches as yet unpublished, is in marked contrast to that of apples examined by Blackman and Parija. In apple, respiration rate rises when the material is transferred to nitrogen to a value higher than the value for air. How far this behaviour is related to the stage of development or to chemical constituents of the respiring cells is not yet clear, but it may be mentioned that Gustafson (18) has found the same rise of respiration rate under anaerobic conditions in the fruit of the tomato. Moreover, the same worker has shown (19) that a number of cacti, including *Carnegia gigantea, Echinocereus fendleri* and *Opuntia versicolor,* evolve as much, or nearly as much, carbon dioxide in absence as in presence of oxygen.

Information with regard to respiratory mechanism has also been sought by an endeavour to find evidence of intermediate products of respiration. If, as is usually supposed to be the case, aerobic and anaerobic respiration are connected, the connection is probably correctly indicated by Blackman's scheme mentioned above according to which, following the earlier views of Pfeffer and Kostytschev, intermediate products are converted either to alcohol and carbon dioxide in absence of oxygen or to carbon dioxide and water in its presence. Investigations on substances produced during anaerobiosis have been made in particular by M. Thomas and J. C. Fidler (13, 14, 15, 44, 45, 46) and by Gustafson (20, 21), the former working with apples, the latter with tomatoes and cacti. It is interesting and important to note that in these structures ethyl alcohol is found not only when oxygen

is absent but when it is present; indeed, in old apples Thomas and Fidler found that alcohol can accumulate even in an atmosphere of 100 per cent. oxygen. Acetaldehyde also appears to be present in all these tissues whether the conditions are aerobic or anaerobic.

Hence Thomas and Fidler are led to conclude that ethyl alcohol can be formed under aerobic as well as anaerobic conditions. In earlier work Thomas had distinguished between two types of what he called zymasis, that is, the splitting of carbohydrate with formation of ethyl alcohol and smaller quantities of acetaldehyde. He called these, respectively, anaerobic zymasis which takes place in absence of oxygen, and carbon dioxide zymasis which takes place in presence of oxygen when carbon dioxide is also present in relatively high concentration. In the former type there is a much higher percentage of acetaldehyde produced than in the latter. In his work on tomatoes Gustafson, however, was unable to find such a difference in the ratio of acetaldehyde to alcohol under the two sets of conditions.

In their later work, Thomas and Fidler conclude that in the apple senescence is accompanied by a change in the metabolism of the fruit whereby considerable amounts of ethyl alcohol and lesser amounts of acetaldehyde accumulate in the fruit kept in air. The higher the oxygen concentration the more the retardation of zymasis but as the fruit grows older the extinction point, that is, the concentration of oxygen required to suppress alcohol production, rises, so that in old apples it is not even reached in 100 per cent. oxygen.

If researches on these lines have not yet solved the problem of the intermediate products of respiration, they have brought to light new and important information and it cannot be doubted that continuance of work on these lines should yield valuable information on the nature of the respiratory mechanism.

Relation of Rate of Carbon Dioxide Output to Rate of Loss of Substrate

The last line of research to be considered here by which information regarding respiratory mechanism has been sought is the correlation of rate of carbon dioxide output with rate of disappearance of the substrate. This has been attempted by H. K. Archbold

and A. M. Barter (1) for apples and by Dastur and Desai (10) for rice seedlings. On F. F. Blackman's scheme, in aerobic respiration of apples, part of the substrate is rebuilt back into the system in the process termed oxidative anabolism and if the substance synthesized is not the substrate, there should be a corresponding divergence between the rate of carbon dioxide evolution and the rate of substrate loss. In apples Archbold and Barter actually found this was the case, the total amount of carbon in the sugar and malic acid lost being 17 to 30 per cent. greater than the carbon in the carbon dioxide evolved. In rice seedlings, however, Dastur and Desai conclude that for both aerobic and anaerobic respiration the carbon dioxide produced is greater than can be accounted for by the loss of carbohydrates. These workers suggest that the excess of carbon dioxide results from oxidation of plant acids produced in protein synthesis, but supporting evidence for this view has still to be obtained.

In conclusion it may be said that the work on respiration which has been carried out during the last five years, although containing nothing particularly spectacular, has materially advanced our knowledge both of the relationship of respiration to external and internal conditions and of the respiratory mechanism. If researches on the latter have succeeded in emphasizing the complexity and our ignorance of the process, they have added very materially to the data which will help in the end to the elucidation of the mechanism of this fundamental process of all living things.

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